"Targeted, liposomal oligonucleotide-polyethylenamine complex for human leukemia treatment"

Abstract

Pharmaceutical industry and methods in cancer therapy especially in recent years, is rapidly changing, due to the need of design of new, more effective therapeutic strategies. Very promising approach to treatment of the neoplastic diseases is antisense gene therapy. Due to the low toxicity of treatment and eliminating not only the symptoms but also the molecular causes of the disease it may represent a breakthrough in cancer therapies. Molecules such as antisense oligodeoxynucleotides (asODNs), small interfering RNA (siRNA, miRNA) or nucleotides with catalytic activity (DNAzymes, ribozymes) can be used to inhibit particular gene expression in monotherapy or in combination with other therapeutic agents to enhance the effectiveness of non-gene therapy. It could be combined with other treatments to enhance therapeutic effect. Most of the diagnosed cases of chronic lymphocytic leukemia (CLL) and B-cell lymphomas are associated with the overexpression of anti-apoptotic *BCL2* gene, which is also a cause of resistance to conventionally used chemotherapeutic agents. Depletion of Bcl-2 protein level that could, itself, prevent the development of cancer or more probably could help sensitize cancer cells to apoptosis inducers. Delivery of a therapeutic DNA or RNA fragment to the target cells *in vivo* requires suitable carrier system.

The main objective of this work was to obtain targeted formulation that combines a liposome and cationic polymers features that could be useful as a carrier for genetic drugs delivery. Proposed here liposomal carrier consist of a core composed of antisense oligonucleotides complexed with synthetic polycation, polyethyleneimine (PEI) encapsulated within negatively charged liposomes modified by polyethylenoglycol (PEG). Carriers are enriched with covalently-bound antibodies recognizing well characterized marker exposed on the surface of leukemia cells.

Construction of the carrier allows to use different types of genetic drugs such as asODN, siRNA, miRNA, DNAzyme to reduce various gene expression, as well as the use of different humanized antibodies or other proteins directing to specific target cells. The model

chosen in this project is a carrier containing asODN sequence directed against the first six codons of anti-apoptotic *BCL2* mRNA. The active targeted drug delivery is achieved by functionalizing carrier with targeting moieties that have affinity toward protein CD20, which is primarily found on the surface of human immune system B cells. Obtained liposome carrier is called t-LP (targeted - Liposomal Coated Polyplex).

Presented here data have shown that the proposed formulation can encapsulate large amounts of nucleic acids and remain stable during incubation with human serum or plasma. Moreover, liposomes maintain stability for at least one year of storage as a suspension and as a freeze-dried powder and effectively protect encapsulated genetic drug from degradation by nucleases. Addition of targeting moieties to the surface of the liposomes make them selective towards target cells whilst did not affect the basic physicochemical properties. In addition, t-LPasODN does not exhibit hemolytic activity and non-specific cytotoxicity.

Immunoliposomes effectively reduced the expression of *BCL2* in cancer cells. Impaired apoptosis related to overexpression of Bcl-2 protein, which is observed in approximately 76% of patients with chronic lympocytic leukemia, is implicated in the resistance to chemotherapy. The analysis by MTT and Alamar Blue cytotoxicity assay showed high efficiency of immunoliposomes in combination with mitoxantrone *in vitro*. Moreover, results obtained by flow cytometry indicate that t-LP induced apoptosis in Daudi cells by reducing the *BCL2* gene expression.

The animal model experiments carried out on mice-engrafted tumor characterized by the presence of specific marker showed high efficiency of the proposed drug formulation against specific tumor development. Liposomes t-LP coated with antibodies were localized in the CD20 tumors derived in mice, in contrast to untargeted liposomes. Developed here lipid carriers based on polyplex (asODN-PEI) backbone additionally equipped with antibodies was shown to be reasonable non-viral vector for specific oligonucleotide transfer into human tumor cells.